

Claims 1-11, 17 and 18 have now been rewritten as new claims 19-36. These claims are patentable for the reasons pointed out below in responding to the prior art rejection of claims 1-11, 17 and 18.

First, claim 19 includes a correction over claim 1 with respect to the definition of the lipid to include in its head group either at least two **acyl**, alkyl or alkenyl chains, or a combination thereof. Claim 19 further clarifies that the lipid head group may contain non-identical chains, e.g. one acyl and one alkyl, at the head group, as long as the two chains each comprise more than 14 carbon atoms.

Support for the above corrections may be found throughout the specification describing several diacylated lipids which may be employed by the method of the invention. One particular diacylated lipid illustrated in the specific example is dioleoyl phosphatidyl ethanolamine (DOPE). Other possible diacylated lipids described are dioleoyl phosphatidyl choline (DOPC), dipalmitoyl phosphatidyl ethanolamine (DPPE), palmitoyloleoyl phosphatidyl choline (POPC), and palmitoyloleoyl phosphatidyl glycerol (POPG).

Claim 19 includes a further correction of claim 1 to refer to sphingolipid. Support for this correction may be found, *inter alia*, on page 7, lines 30-33 reciting that "The

steroid or lipid, such as phospholipid or **sphingolipid**, may be conjugated to the fluorophore by known synthetic methods."

Newly added claims 23 and 24 are supported by original claims 15 and 16 which were removed from the claims as a result of the Restriction Requirement. Additional support may be found in the description, *inter alia*, in the passage abridging pages 2 and 3.

Claims 1-11, 17 and 18 have been rejected as obvious under §103 from the Zuidam et al publication (Zuidam) in view of Gee et al USP 5,830,912 (Gee). This rejection is respectfully traversed.

The primary reference, i.e. the publication of Zuidam and Barenholz, is of course somewhat related to the present invention, noting that Dr. Barenholz is the first named inventor of the present application. However, contrary to what is stated in the rejection, such publication (hereinafter simply "Zuidam") does not describe the method of the instant invention. In particular, Zuidam does not disclose the stable incorporation of the probe at the surface. In this regard, the present invention specifically requires that the probe be stably incorporated at the lipid surface. The term "stably incorporated" is defined at page 6 (emphasis added) :

"Stably incorporated", with respect to a probe incorporated in a lipid bilayer, indicates that the probe **has a low desorption rate from the bilayer**. This can be accomplished by employing an anchoring hydrophobic group such as a steroid or a dialkyl or dialkenyl lipid having carbon chains 14 carbons or greater in length.

This means that the probe employed by the present invention must be such that changes at the surface, for example as a result of binding of species thereat, will have minimal impact on the desorption rate from the surface.

As indicated throughout the specification, the stable incorporation of the probe at the surface may be achieved by attaching a fluorophore to a steroid or to a head group of a lipid having e.g. at least two acyl, alkyl or alkenyl chains, each chain comprising at least 14 carbon atoms in length or to a head group of a sphingolipid.

To the contrary, Zuidam teaches a system in which the **incorporation of the fluorophore is unstable**. As stated on page 1 (lines 24-27) of the specification, the use of probes containing the pH-dependent fluorescent molecule 7-hydroxycoumarin (which is not modified with hydrophobic groups, as in the instant invention) is hampered by the **instability** of the probe in the bilayer.

Zuidam makes use of 4-heptadecyl-7-hydrocoumarin (HC) as the fluorophore. This publication clearly teaches that the incorporation of fluorophores at a lipid layer is

unstable, and that HC dissociates (as a result of its ionization) from the lipid layers as a result of binding of the tested species, being in that case a plasmid DNA. This publication in fact presents a study concerning **the changes in the dissociation (or ionization) degree of HC in the lipid layers at different DNA concentrations, as a result of changes in the electrostatic properties of the water/lipid interface**. The changes are monitored with time by measuring the value of excitation ratios at different wavelengths (see page 117, under subtitle 3.1).

Against this, the present invention discloses a method in which it is essential that the probe (fluorophore) be stably incorporated at the surface, such that when species bind or dissociate from the surface, changes in a fluorescent property at the surface environment are not as a result of dissociation of the probe from the surface.

Thus, the present invention provides a method which enables observing, in a stable and reproducible manner, changes in the environment of a surface not necessarily dependent on interactions of the probe with a specific molecule, i.e. both specific and non-specific interactions at the surface can be detected. This is not disclosed, taught or even remotely inferred by Zuidam.

Gee merely teaches 6,8-difluoro-7-hydroxycoumarin derivatives. Compound 68, referred to in the rejection, contains a dialkyl phospholipid moiety in addition to the fluoro substituents. Gee does not suggest or infer the significance of modifying 7-hydroxycoumarin with hydrophobic groups for achieving a stable and reproducible method for determining changes in an environment of a surface as a result of binding of species thereat, which is independent of the specific interactions between the species and the probe (as per the discussion above).

Thus, not only does Gee not make up for the aforementioned deficiencies of Zuidam, but Gee has not even been cited for ant teaching of stability in the claimed environment. It is a leap to go from a consideration of Gee, while aware of Zuidam, to come up with applicants' invention. The person of ordinary skill in the art, looking at Zuidam together with Gee, would not have come up with the present invention.

Applicants also do not accept the proposition that the person of ordinary skill in the art would even wish to attempt to combine these diverse references. The prior art provides no reasonable expectation that the proposed substitution, even if apparent to the skilled person, would achieve applicants' surprising results as discussed above.

Thus, it could not have been predicted or foreseen that to proceed as applicants have done would enable the observation, in a stable and reproducible manner, of changes in the environment of a surface not necessarily dependent on interaction of the probe with a specific molecule.

Applicants respectfully request withdrawal of the rejection based on §103.

Favorable reconsideration and allowance are earnestly solicited.

Respectfully submitted,

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